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(54) Title: METHOD AND APPARATUS FOR FABRIC	CATING	MICROARRAYS OF BIOLOGICAL SAMPLES

(57) Abstract

A method and apparatus for forming microarrays of biological samples on a support are disclosed. The method involves dispensing a known volume of a reagent at each of a selected array position, by tapping a capillary dispenser on the support under conditions effective to draw a defined volume of liquid onto the support. The apparatus is designed to produce a microarray of such regions in an automated fashion.

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METHOD AND APPARATUS FOR FABRICATING MICROARRAYS OF BIOLOGICAL SAMPLES

Field of the Invention

This invention relates to a method and apparatus for fabricating microarrays of biological samples for large scale screening assays, such as arrays of DNA samples to be used in DNA hybridization assays for genetic research and diagnostic applications.

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References

Abouzied, et al., Journal of AOAC International 77(2):495-500 (1994).

Bohlander, et al., Genomics 13:1322-1324 (1992).

Drmanac, et al., Science 260:1649-1652 (1993).

Fodor, et al., Science 251:767-773 (1991).

Khrapko, et al., DNA Sequence 1:375-388 (1991).

Kuriyama, et al., <u>An ISFET BIOSENSOR</u>, <u>APPLIED BIOSENSORS</u> (Donald Wise, Ed.), Butterworths, pp. 93-114 (1989).

Lehrach, et al., <u>Hybridization Fingerprinting in Genome Mapping and Sequencing, Genome Analysis, Vol 1</u> (Davies and Tilgham, Eds.), Cold Spring Harbor Press, pp. 39-81 (1990).

Maniatis, et al., <u>Molecular Cloning</u>, <u>A Laboratory</u>

25 <u>Manual</u>, Cold Spring Harbor Press (1989).

Nelson, et al., Nature Genetics <u>4</u>:11-18 (1993).

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Pirrung, et al., U.S. Patent No. 5,143,854 (1992).
Riles, et al., Genetics 134:81-150 (1993).
Schena, M. et al., Proc. Nat. Acad. Sci. USA
89:3894-3898 (1992).

5 Southern, et al., Genomics <u>13</u>:1008-1017 (1992).

Background of the Invention

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A variety of methods are currently available for making arrays of biological macromolecules, such as arrays of nucleic acid molecules or proteins. One method for making ordered arrays of DNA on a porous membrane is a "dot blot" approach. In this method, a vacuum manifold transfers a plurality, e.g., 96, aqueous samples of DNA from 3 millimeter diameter wells to a porous membrane. A common variant of this procedure is a "slot-blot" method in which the wells have highly-elongated oval shapes.

The DNA is immobilized on the porous membrane by baking the membrane or exposing it to UV radiation. This is a manual procedure practical for making one array at a time and usually limited to 96 samples per array. "Dot-blot" procedures are therefore inadequate for applications in which many thousand samples must be determined.

A more efficient technique employed for making ordered arrays of genomic fragments uses an array of pins dipped into the wells, e.g., the 96 wells of a microtitre plate, for transferring an array of samples to a substrate, such as a porous membrane. One array includes pins that are designed to spot a membrane in a staggered fashion, for creating an array of 9216 spots in a 22 × 22 cm area (Lehrach, et al., 1990). A limitation with this approach is that the volume of DNA spotted in each pixel of each array is highly variable.

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In addition, the number of arrays that can be made with each dipping is usually quite small.

An alternate method of creating ordered arrays of nucleic acid sequences is described by Pirrung, et al. (1992), and also by Fodor, et al. (1991). The method involves synthesizing different nucleic acid sequences at different discrete regions of a support. method employs elaborate synthetic schemes, and is generally limited to relatively short nucleic acid sample, e.g., less than 20 bases. A related method has been described by Southern, et al. (1992).

Khrapko, et al. (1991) describes a method of making an oligonucleotide matrix by spotting DNA onto a thin layer of polyacrylamide. The spotting is done manually with a micropipette.

None of the methods or devices described in the prior art are designed for mass fabrication of microarrays characterized by (i) a large number of micro-sized assay regions separated by a distance of 50-200 microns or less, and (ii) a well-defined amount, typically in the picomole range, of analyte associated with each region of the array.

Furthermore, current technology is directed at performing such assays one at a time to a single array of DNA molecules. For example, the most common method for performing DNA hybridizations to arrays spotted onto porous membrane involves sealing the membrane in a plastic bag (Maniatas, et al., 1989) or a rotating glass cylinder (Robbins Scientific) with the labeled 30 hybridization probe inside the sealed chamber. arrays made on non-porous surfaces, such as a microscope slide, each array is incubated with the labeled hybridization probe sealed under a coverslip. These techniques require a separate sealed chamber for

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each array which makes the screening and handling of many such arrays inconvenient and time intensive.

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Abouzied, et al. (1994) describes a method of printing horizontal lines of antibodies on a nitrocellulose membrane and separating regions of the membrane with vertical stripes of a hydrophobic material. Each vertical stripe is then reacted with a different antigen and the reaction between the immobilized antibody and an antigen is detected using a standard ELISA colorimetric technique. Abouzied's technique makes it possible to screen many onedimensional arrays simultaneously on a single sheet of nitrocellulose. Abouzied makes the nitrocellulose somewhat hydrophobic using a line drawn with PAP Pen (Research Products International). However Abouzied does not describe a technology that is capable of completely sealing the pores of the nitrocellulose. The pores of the nitrocellulose are still physically open and so the assay reagents can leak through the hydrophobic barrier during extended high temperature incubations or in the presence of detergents which makes the Abouzied technique unacceptable for DNA hybridization assays.

Porous membranes with printed patterns of hydrophilic/hydrophobic regions exist for applications such as ordered arrays of bacteria colonies. QA Life Sciences (San Diego CA) makes such a membrane with a grid pattern printed on it. However, this membrane has the same disadvantage as the Abouzied technique since reagents can still flow between the gridded arrays making them unusable for separate DNA hybridization assays.

Pall Corporation make a 96-well plate with a porous filter heat sealed to the bottom of the plate. These plates are capable of containing different